

epithelial tumor cells that underwent a transient dedifferentiation associated with EMT rather than cancer stem cells that pre-exist as a subpopulation of the primary tumor. YB-1 gain or loss of translational control could potentially be involved in the regulation of both EMT and MET, respectively.

These studies raise the question: is YB-1 a target for therapeutic intervention in metastasis? As the authors point out, direct targeting of YB-1 might prove to be futile or even counterproductive since YB-1 could be an important factor in

keeping cells dormant after they metastasize.

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Agonizing Integrin Antagonists?

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A recent study published in *Nature Medicine* reports that low-dose treatment with RGD-mimetic integrin inhibitors may paradoxically enhance angiogenesis and tumor growth. This work implies that delivery of these agents should be redesigned in order to avoid nanomolar plasma concentrations and to improve their efficacy to treat human cancers.

Integrins mediate cell-cell and cell-matrix interactions to “integrate” extracellular cues with intracellular signaling pathways and to promote a wide array of biological responses. During development, tissue remodeling, and in various disease conditions, integrin-mediated cell migration/invasion occurs via transient binding and release of the extracellular matrix (ECM). Thus, local microenvironments containing substrate and soluble protein fragments provide situational cues to integrins which guide cell behavior. Several integrins, including $\alpha v \beta 3$ and $\alpha v \beta 5$, recognize the arginine-glycine-aspartic acid (RGD) sequence shared by a number of extracellular matrix ligands. Accordingly, RGD-mimetic peptides or small molecules bind to these integrins on the surface of cells to block specific αv integrin-mediated signaling pathways and act as anticancer and antiangiogenic agents.

Reynolds and colleagues recently reported in *Nature Medicine* that low nanomolar concentrations of RGD mimetics

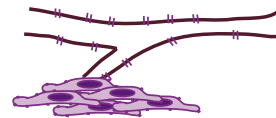
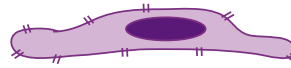
may actually stimulate tumor growth and angiogenesis (Reynolds et al., 2009). These conclusions were drawn from examination of B16F0 melanoma or LLC lung carcinoma cells grown on the flanks of syngeneic C57BL6 mice treated systemically with a cyclic RGD peptide (ciclegitide) or a small molecule RGD mimetic (S 36578). Since the RGD mimetics did not increase tumor growth or vascularization in integrin $\beta 3/\beta 5$ double knockout mice, the authors concluded that low doses of integrin antagonists promoted tumor growth by acting on host endothelial cells. While these findings may seem surprising, several independent groups reported in the early 1990s that nanomolar concentrations of soluble ECM proteins could induce chemotaxis (Aznavorian et al., 1990) and that the chemotaxis induced specifically by RGD-containing fibronectin fragments could be overcome by millimolar concentrations of RGD-containing peptides (Odekon et al., 1991). As a rationale for their experiments, Rey-

nolds et al. also pointed to work by Legler and coworkers who found that cyclic RGD-peptides have a biphasic effect on $\alpha v \beta 3$, with an antagonistic phase at high concentrations and an agonistic phase at low concentrations (Legler et al., 2001). Thus, it has been known for years that low concentrations of soluble antagonists will agonize or “superactivate” (Legler et al., 2001) integrins (Figure 1). Nonetheless, in their new study, Reynolds et al. have extended these previous findings to confirm that integrin activation by low-dose antagonists does occur in endothelial cells and may be relevant for angiogenesis among subcutaneously growing tumors.

The authors commented in the paper that integrin antagonism as an antiangiogenic therapy has been largely unsuccessful in man and that any observed efficacy is probably due to direct action on tumor cells. While it is generally true that many antiangiogenic strategies for cancers have performed below

IN VITRO:
Tumor or endothelial cells
expressing α_v integrins

IN VIVO:
Tumors & angiogenic blood
vessels expressing α_v integrins



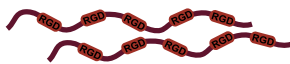
Effect of soluble integrin ligand:

Sustained low nM dose:
Integrin activation

Higher doses:
Integrin antagonism

Modulation of integrin activation:

RGD-containing ECM proteins



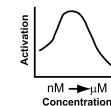
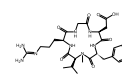
RGD-containing soluble protein fragments

Tumor cells may encounter pools
of soluble ECM fragments
that stimulate migration
(Aznavorian et al., 1990)



RGD-mimetic drugs

Superactivation of integrin $\alpha_v\beta_3$
by low antagonist concentrations
(Legler et al., 2001)



Recycling of growth factor receptors & integrins

Rab-coupling protein coordinates
EGFR1 & $\alpha_5\beta_1$ recycling in tumor cells
(Caswell et al., 2008)

Rab4A drives VEGFR2 & $\alpha_v\beta_3$
recycling in endothelial cells
(Reynolds et al., 2009)

Angiogenesis & Tumor Growth

Figure 1. Balance of Integrin Activation Regulates Cell Movement between Tissue Microenvironments

Activity of α_v integrins can be modulated by binding to soluble integrin ligand, leading to changes in cell behavior. High doses of RGD-mimetic integrin inhibitors act as integrin antagonists and antiangiogenic agents, whereas low doses of the same drugs paradoxically activate α_v integrins to promote migration, invasion, and angiogenesis. The signals produced by endogenous RGD-containing ECM proteins and soluble fragments can thereby be outcompeted by exposure to μ M doses of RGD-mimetic drugs.

expectation, cilengitide as a single therapy has shown efficacy and antiangiogenic activity in glioma patients (Nabors et al., 2007) and achieved stable disease in late-stage glioblastoma, a significant improvement over existing treatment strategies. In addition, cilengitide in combination with chemotherapies or other agents is currently being tested in ongoing clinical trials. Reynolds et al. further show that antibody-mediated blockade of VEGFR2 function (using DC101) suppresses the ability of nanomolar concentrations of integrin antagonists to promote angiogenesis and tumor growth. Based on these findings, the authors speculate that the antiangiogenic efficacy of integrin antagonism could be improved by combination with VEGFR antagonism. While anti-VEGF (i.e., bevacizumab) as a single therapy produces little or no efficacy in man, approaches that target the VEGF pathway serve to

normalize vasculature, thereby facilitating drug delivery to the tumor (Fukumura and Jain, 2007; Greenberg et al., 2008). Future studies must optimize whether anti-angiogenic strategies should be employed as single or combination therapies to treat specific cancers.

Mechanistically, Reynolds et al. report that low doses of integrin antagonists induce angiogenesis by enhancing VEGF-stimulated migration of endothelial cells, which depends on their capacity to promote Rab4A-mediated recycling of VEGFR2 and $\alpha_v\beta_3$ integrin. Although blood vessels in normal tissues do not express $\alpha_v\beta_3$, tumor-associated vessels express high levels of this integrin. Thus, the use of cultured endothelial cells to study recycling kinetics of VEGFR2 or $\alpha_v\beta_3$ may not be entirely representative of the in vivo situation. Nevertheless, the authors point out that cilengitide has recently been shown to promote tumor

cell invasion by altering recycling kinetics of integrin $\alpha_5\beta_1$ and EGFR (Caswell et al., 2008). Thus, integrin antagonism may modulate cell migration in general by promoting integrin and growth factor recycling in tumor cells and endothelial cells alike (Figure 1). Future work is required to understand how various other cell types involved with tumor progression (such as fibroblasts or smooth muscle cells) may respond to low or high doses of integrin antagonists.

To directly assess the impact of integrin antagonism on angiogenic sprouting, the authors used an aortic ring assay to demonstrate that low doses of RGD mimetics enhance endothelial sprouting into a 3D extracellular matrix, independent of any effects on tumor cells. This model yielded an interesting finding: exposure to low doses of the integrin antagonist impaired the antiangiogenic response to subsequently applied higher doses. The

authors speculate that, in patients, bolus injections resulting in fluctuations between high and low concentrations of integrin antagonists might undermine the efficacy of such inhibitors. However, this hypothesis needs to be tested using more relevant preclinical models, as the *ex vivo* sprouting of aortic ring explants only poorly approximates the complicated pharmacokinetics or microenvironment associated with systemic dosing of patients with such drugs.

It should be pointed out that Reynolds et al. used an osmotic minipump to deliver constant low nM doses of the drug in order to maintain its low steady-state level in mice with flank tumors. It remains to be seen how chronic low-dose administration of integrin antagonists is relevant to the much higher average concentrations of the same drugs which have been reported to produce clinical activity in man (Nabors et al., 2007). It is also not clear how plasma concentrations of integrin antagonists correlate to the varying concentrations within different microenvironments of a tumor. Furthermore, there are endogenous soluble integrin ligands such as tumstatin, endostatin, or angios-

tatin that may function cooperatively with RGD mimetics, making the effective concentration of soluble integrin antagonists *in vivo* difficult to interpret. As typically used for human trials, bolus administration of integrin antagonists may provide a better clinical correlate than the experimental conditions as defined by Reynolds et al. The use of such inhibitors might also be better applied to either spontaneous tumors or those that are orthotopically implanted, since previous mouse studies have revealed that cilengitide blocks angiogenesis and glioma progression in the orthotopic environment of the brain but is ineffective on these cells growing in the same animals as a subcutaneous xenograft (MacDonald et al., 2001). Although RGD mimetics might be particularly suited for the brain microenvironment, these agents may also provide significant benefit in other tumors depending on local tissue cues. Regardless, the host response to the tumor microenvironment is currently a hot topic in cancer research, and integrin signaling plays a key role in determination of cell fate when exposed to different extracellular matrix components.

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